UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF **C**HEMICAL **S**AFETY
AND **P**OLLUTION **P**REVENTION

September 7, 2017

MEMORANDUM

SUBJECT: Review of revised test protocol for an acute *Daphnia magna* toxicity test with NSPW-L30SS

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BACKGROUND

Poly-Technical Solutions submitted a revised draft protocol for a *Daphnia magna* 48-Hour Acute Toxicity Test with NSPW-L30SS (August 22, 2017 email from K. Schaumburg to S. Murasaki USEPA/OCSPP/OPP/AD/RMB I), which contains silver nanoparticles, for Agency review. The modifications in this revised draft address a number of issues identified by RASSB scientists in a July 27,

2017 Memorandum reviewing the first draft of this protocol[1,2]. While some recommendations not elected to be included in the revised protocol will not affect the acceptability of the study, there are critical components that if not addressed adequately will affect study acceptability. The Agency has provided a discussion of these issues in the following section.

DISCUSSION

The major critical issues with the revised study protocol include the following areas: lack of inclusion of a nanoparticle reference material and lack of critical nanomaterial-specific analytical methods as discussed in the Agency's July 27, 2017 protocol review Memorandum[2]. Detailed discussions for each of these are provided in the following bulleted sections.

- **Test substance preparation and exposure study type: combine study with a kinetic dissolution/stability study in bioassay medium instead of a solubility study (Section B.4.b)**

No kinetic dissolution/stability study was included or referenced in the revised protocol, only a solubility study (Section B.4.b.) with no details on how it is to be conducted or how solubility in this case is being defined. Because the nanosilver-silica composite particle diameter size (average 0.32 microns[3]) is within the operational definition of dissolved using conventional methods of either centrifugation (1E+05 to 4E+05 $m \cdot s^{-2}$ for 30 minutes) or filtration (0.45 micron) as discussed in the previous review[2], all of NSPW-L30SS potentially will be identified as "soluble" even if it was present as NSPW-L30SS. For risk assessment purposes, all the forms of silver present throughout the duration of the study need to be identified and quantified, a typical guideline solubility study will not provide adequate data.

- **Include appropriate protocols for characterization and analysis of the nanomaterial and transformation products (e.g. ionic silver) and verify in additional test doses (Sections B.4.c. and B.5.e.)**

The Pretest Concentration Confirmation focuses on analysis of total silver concentration in water as verification of the identity, stability, and exposure to NSPW-L30SS throughout the study. As pointed out in the previous protocol review[2], measurement of total silver alone is not an appropriate method for verifying the stability and exposure levels to a metal nanomaterial.

The proposed Volhard's Thiocyanate Method is a titration method with a detection limit typically in the ppm range and relies on reacting ionic silver with chlorides. There are a number of concerns with use of this method. The silver nanoparticles present in the NSPW-L30SS material may not have the necessary surface charge to form the halide complex, may not dissociate readily, and may be prevented from readily reacting due to surface encapsulation. Should the nanoparticle ionize, it is unclear whether the test would distinguish between ionic silver and the silver nanoparticles. It would be necessary to provide validation of this method for adequacy in distinguishing between and characterizing the concentration of: total silver; ionic silver; dissolved silver; and silver nanoparticles, using both a reference silver nanomaterial and the NSPW-L30SS product at test concentrations, not just using silver nitrate.

Since acute toxicity of silver in silver nitrate to *D. magna* in moderately hard reconstituted water (80 – 90 mg/L as $CaCO_3$) is on the order of 0.2 to 1.2 µg/L on a total basis and 1.8 to 187 µg/L total silver for silver nanoparticle formulations reviewed by the Agency[4], and is lower in both cases on a dissolved silver

[1] MRID 50301209: Protocol for study
[2] DP Barcode 441388: July 27, 2007 Memorandum from D. Randall and A. Byro USEPA/OCSPP/OPP/AD/RASSB to J. Herbert, S. Murasaki, Z. Bain USEPA/OCSPP/OPP/AD/RMB I, Re: Protocol Review of an acute *Daphnia magna* toxicity test with NSPW-L30SS, 6 pp.
[3] USEPA, 2015. Registration Decision for NSPW-L30SS. May 5, 2015. 83 pp.
[4] Table 17 in USEPA, 2015. Registration Decision for NSPW-L30SS.

basis, the Volhard method does not appear to be sensitive enough for quantification of the silver as total, ionic, or dissolved in this range. Additionally, a total silver basis does not inform the Agency regarding if NSPW-L30SS is stable throughout the duration of the study (48 hours) if static conditions are used, or even for 24 hours if static renewal conditions are used. A total silver analysis with agreement within ± 80% (Section B.4.c.) only confirms that the total silver content has not changed which would not be unexpected because silver does not volatilize and as an element is not expected to dissipate via abiotic or biotic degradation/transformation pathways. If the silver in the NSPW-L30SS dissolves, e.g., increased ionic silver or dissolved or nanosilver chloride complexes over test duration or even if silver chloride concentration increases, then NSPW-L30SS is not stable. No optical methods were identified for characterizing and quantifying particle dispersion and stability throughout the duration of the test.

Some standardized methods for characterizing nanoparticles (such as ISO 22412:2017) may be combined with complementary methods such as transmission electron microscopy and/or scanning electron microscopy. This would also allow the lab to evaluate the tendency for the nanoparticle to agglomerate or aggregate, particularly at higher concentrations, which may affect the experiment. If the particles do not aggregate, it would additionally allow the calculation of an average number of nanoparticles which could be used to confirm other concentration measurements.

References were provided in the July 27, 2017 Memorandum[2] that could aid in selection of appropriate methodology. Changes to the nanomaterials need to be accurately measured so as to lead to accurate dosing metrics (Diamond et al., 2009; Petersen et al., 2014; Kennedy et al.,2010; Zook et al., 2015). Without appropriate analytical methods, the Agency will not know to what exactly *D. magna* has been exposed throughout the test and in what quantities.

The revised protocol only includes dose verification at the highest dose concentration (Section B.5.e). Because nanoparticles may act differently under different dilutions (e.g., dissolve more rapidly, agglomerate or dissaggregate, measurement at only the highest test concentration for NSPW-L30SS will be insufficient to verify exposure to NSPW-L30SS at other dilutions.

- **Inclusion of a nanoparticle reference material in the study (Section B.4.i)**

No metal nanoparticle reference material test was included in the revised protocol. Correct preparation of nanomaterials for aquatic testing is difficult, and even facilities with experience preparing such materials include a nanoparticle reference material as verification. Without inclusion there is no way the testing facility can validate that the preparation methods used resulted in creation of a dispersed working stock solution and dispersed spiked suspensions in aqueous bioassay media. Non homogenous dispersions of nanomaterials have led to high variability in toxicity results (Coleman, Kennedy, and Harmon, 2015; Diamond et al., 2009).

- **Include histological sampling and observations (Section B.6)**

No histological sampling was added to the revised protocol as recommended by the Agency in its July 27, 2017 protocol review[2]. Without such information to document that any released silver nanoparticles or the nanosilica composite is not taken up within the organism, the Agency will assume that NSPW-L30SS and any released nanosilver particles are the forms taken up within the organism and which can then be transferred up the food chain.

Other minor recommendations:

- Section B.3. Contaminants. No specifics are provided on how laboratory personnel will minimize any possible silver contamination, or dust particulate introduction.

- Section B.5.a. Dosing Concentrations. For pesticides the limit dose is 1000 mg/L as the active ingredient, not test substance.

REFERENCES

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